CLAIMS

We claim:

1. A method for detecting a target sequence comprising a first and second target domain in a sample, said method comprising:

a) hybridizing said target sequence to a precircle probe to form a first hybridization complex, said precircle probe comprising:

i) a first targeting domain;

ii) a second targeting domain;

iii) at least a first universal priming site; and

iv) a cleavage site;

wherein said first and second targeting domains hybridize to said first and second target domains;

b) contacting said first hybridization complex with a ligase to form a closed circular probe;

c) cleaving said closed circular probe at said cleavage site to form a cleaved probe;

d) amplifying said cleaved probe to form a plurality of amplicons; and

e) detecting said amplicons to detect the presence of said target sequence in said sample.

2. A method according to claim 1 wherein said amplifying is done by contacting said cleaved probe with:

a) at least a first universal primer;

b) an extension enzyme; and

c) NTPs.

3. A method according to claim 2 wherein said extension enzyme is a polymerase.

4. A method according to Claim 1, wherein said precircle probe further comprises a second universal priming site, and said second contacting step further comprises contacting said cleaved probe with a second universal primer.

5. A method according to Claim 4, wherein said cleavage site is between said first and second universal priming site.

6. A method according to Claim 1, wherein said target sequence further comprises a gap domain between said first and second target domains and said method further comprises the additional step of contacting said first hybridization complex with an extension enzyme and at least one interrogation NTP prior to forming said closed circular probe.

7. A method according to Claim 1, wherein said target sequence further comprises a gap domain between said first and second target domains and said method further comprises the additional step of contacting said first hybridization complex with at least one gap oligonucleotide prior to forming said closed circular probe, said gap oligonucleotide having a nucleic acid sequence perfectly complementary to said gap domain, wherein detecting said amplicons identifies said gap domain.

8. A method according to Claim 1, wherein said target sequence further comprises a gap domain between said first and second target domains and said precircle probe further comprises a 3' or 5' most detection domain comprising one or more nucleic acids perfectly complementary to said gap domain, wherein detecting said amplicons identifies said gap domain.

9. A method according to Claim 8, wherein said detection domain is joined to said second targeting domain.

10. A method according to Claim 8, wherein said gap domain is from one to about 1000 nucleotides.

11. A method according to Claim 8, wherein said gap domain corresponds to a single nucleotide polymorphism in said target sequence.

12. A method according to Claim 1, comprising the additional step of digesting any linear precircle probes prior to cleaving said closed circular probe.

13. A method according to Claim 1 further comprising degrading any dNTPs prior to the addition of said interrogation dNTPs.

14. A method according to claim 13 wherein said degrading is done with apyrase.

15. A method according to Claim 1, wherein said cleavage site is a uracil and said cleavage step comprises contacting said closed circular probe with uracil-N-glycolylase.

16. A method according to Claim 1, wherein said cleavage site is a restriction site and said cleavage step comprises contacting said closed circular probe with a restriction enzyme.

17. A method according to Claim 1, wherein said at least one of said universal primers is labeled.

18. A method according to Claim 1, wherein at least one of said NTPs is labeled.

19. A method according to Claim 17 or 18, wherein said label comprises biotin and said method further comprises the additional step of separating biotinylated amplicons prior to said amplification step.

20. A method for detecting a target sequence in a sample, said target sequence comprising a first and second target domain and a gap domain between said first and second target domains, said method comprising:

a) hybridizing at least one of a plurality of precircle probes to said target sequence to form a plurality of first hybridization complexes, said precircle probes each comprising:

i) a first targeting domain;

ii) a second targeting domain;

iii) a detection domain;

iv) at least a first universal priming site;

v) a cleavage site; and

vi) a barcode sequence;

wherein said plurality of first and second targeting domains are complementary to said plurality of first and second target domains and said gap domain will hybridize to at least one of said plurality of detection domains;

b) contacting said plurality of first hybridization complexes with a ligase to form a plurality of closed circular probes;

c) cleaving said plurality of closed circular probes at said cleavage sites to form a plurality of cleaved probes;

d) amplifying said cleaved probes to form amplicons; and

e) detecting the presence of said amplicons to detect the presence of said plurality of target sequences in said sample.

21. A method according to claim 20 wherein said amplifying is done by contacting said cleaved probe with:

a) at least a first universal primer;

b) an extension enzyme; and

c) NTPs.

22. A method according to claim 21 wherein said extension enzyme is a polymerase.

23. A method according to Claim 20, wherein said plurality of precircle probes each further comprise a second universal priming site, and said contacting step further comprises contacting said plurality of cleaved probes with a second universal primer.

24. A method according to Claim 22, wherein said cleavage site is between said first and second universal priming site.

25. A method according to Claim 20, wherein said target sequence further comprises a gap domain between said first and second target domains and said plurality of precircle probes each comprise a unique barcode and further comprise a 3' or 5' most detection domain comprising one or more nucleic acids complementary to said gap domain, wherein detecting said barcode identifies said gap domain.

26. A method for detecting in a sample a plurality of target sequences, wherein each of said plurality of target sequences comprises first and second target domains, said method comprising:

a) hybridizing said plurality of target sequences to a plurality of precircle probes to form a plurality of first hybridization complexes, each of said precircle probes comprising:

i) a first targeting domain;

ii) a second targeting domain;

iii) at least a first universal priming site;

iv) a cleavage site; and

v) a barcode;

wherein said plurality of first and second targeting domains hybridize to said plurality of first and second target domains;

b) contacting said plurality of first hybridization complexes with a ligase to form a plurality of closed circular probes;

c) cleaving said plurality of closed circular probes at said cleavage sites to form a plurality of cleaved probes;

d) amplifying said cleaved probes to form amplicons; and

e) detecting the presence of said amplicons to detect the presence of said plurality of target sequences in said sample.

27. A method according to claim 26 wherein said amplifying is done by contacting said cleaved probe with:

a) at least a first universal primer;

b) an extension enzyme; and

c) NTPs.

28. A method according to claim 27 wherein said extension enzyme is a polymerase.

29. A method according to Claim 26, wherein said plurality of precircle probes each further comprise a second universal priming site, and said contacting step further comprises contacting said plurality of cleaved probes with a second universal primer.

30. A method according to Claim 29, wherein said cleavage site is between said first and second universal priming site.

31. A method according to Claim 26, wherein said plurality of target sequences each further comprise a gap domain between said first and second target domains, and said method further comprises the additional step of contacting said plurality of first hybridization complexes with a polymerase and at least one dNTP prior to contacting said complexes with said ligase to form a plurality of said closed circular probes.

32. A method according to Claim 26, wherein said plurality of target sequences each further comprise a gap domain between said first and second target domains, and said method further comprises the additional step of contacting said plurality of first hybridization complexes with at least one gap oligonucleotide prior to forming said plurality of closed circular probes, said gap oligonucleotide having a nucleic acid sequence complementary to at least one of said plurality of gap domains, wherein detecting said amplicons identifies said gap domains.

33. A method according to Claim 26, wherein said plurality of target sequences each further comprise a gap domain between said first and second target domains, and wherein said plurality of precircle probes each comprise a unique barcode and further comprise a detection region comprising one or more nucleic acids complementary to at least one of said gap domains.

34. A method for identifying the base at a detection position in a target sequence comprising a first and second target domain separated by a gap domain, said gap domain comprising said detection position, said method comprising:

a) hybridizing said target sequence to a precircle probe to form a first hybridization complex, said precircle probe comprising:

i) a 5' first targeting domain;

ii) a 3' second targeting domain;

iii) at least a first universal priming site; and

iv) a cleavage site;

wherein said first and second targeting domains hybridize to said first and second target domains;

b) contacting said first hybridization complex with a polymerase and at least one interrogation dNTP to form an extended precircle probe;

c) contacting said first hybridization complex comprising said extended precircle probe and said target sequence with a ligase to form a closed circular probe;

d) cleaving said closed circular probe at said cleavage site to form a cleaved probe;

e) amplifying said cleaved probe to form a plurality of amplicons;

f) detecting the presence of said amplicons to detect the presence of said target sequence in said sample.

35. A method according to Claims 34, further comprising degrading any dNTPs prior to the addition of said interrogation dNTPs.

36. A method according to claim 35 wherein said degrading is done with apyrase.

37. A method for amplifying a target sequence comprising a first and second target domain in a sample, said method comprising:

a) hybridizing said target sequence to a precircle probe to form a first hybridization complex, said precircle probe comprising:

i) a first targeting domain;

ii) a second targeting domain;

iii) at least a first universal priming site; and

iv) a cleavage site;

wherein said first and second targeting domains hybridize to said first and second target domains;

b) contacting said first hybridization complex with a ligase to form a closed circular probe;

c) cleaving said closed circular probe at said cleavage site to form a cleaved probe; and

d) amplifying said cleaved probe.

38. A method according to Claim 37, wherein said target sequence further comprises a gap domain between said first and second target domains, and said method further comprises the additional step of contacting said first hybridization complex with a polymerase and at least one NTP prior to contacting said complex with said ligase to form said closed circular probes.

39. A method according to any of Claims 37, comprising the additional step of digesting any linear precircle probes prior to cleaving said closed circular probe.

40. A method for detecting a target sequence comprising a first and second target domain in a sample, said method comprising:

a) hybridizing said target sequence to a precircle probe to form a first hybridization complex, said precircle probe comprising:

i) a first targeting domain;

ii) a second targeting domain; and

iii) at least a first universal priming site;

wherein said first and second targeting domains hybridize to said first and second target domains;

b) contacting said first hybridization complex with a ligase to form a closed circular probe;

c) contacting said closed circular probe at least a first universal primer, an extension enzyme and NTPs to form an extension product;

d) amplifying said extension product to form amplicons; and

e) detecting said amplicons to detect the presence of said target sequence in said sample.

41. A method according to claim 40 wherein said amplifying is done by contacting said extension product with:

a) at least a first universal primer;

b) an extension enzyme; and

c) NTPs.

42. A method according to Claim 40, wherein said target sequence further comprises a gap domain between said first and second target domains and said method further comprises the additional step of contacting said first hybridization complex with an extension enzyme and at least one interrogation NTP prior to forming said closed circular probe.

43. A method according to claim 40, wherein said target sequence further comprises a gap domain between said first and second target domains and said method further comprises the additional step of contacting said first hybridization complex with at least one gap oligonucleotide prior to forming said closed circular probe, said gap oligonucleotide having a nucleic acid sequence perfectly complementary to said gap domain, wherein detecting said amplicons identifies said gap domain.

44. A method according to Claim 40, wherein said target sequence further comprises a gap domain between said first and second target domains and said precircle probe further comprises a 3' or 5' most detection domain comprising one or more nucleic acids perfectly complementary to said gap domain, wherein detecting said amplicons identifies said gap domain.

45. A method according to Claim 40, wherein said precircle probe further comprises at least one cleavage site and said closed circular probe is cleaved prior to amplifying said extension product.

46. A method according to Claim 41, wherein said at least one cleavage site comprises uracil and said cleavage step comprises contacting said closed circular probe with uracil-N-glycolylase.

47. A method according to Claim 45, wherein said cleavage site is a restriction site and said cleavage step comprises contacting said closed circular probe with a restriction enzyme.